

## Behavioral and Physiological Response of *Musca domestica* to Colored Visual Targets

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**ABSTRACT** A better understanding of the visual attraction of house flies to colors and patterns is needed to improve fly trap performance. This study combined physiological responses measured with electroretinogram studies of the house fly's compound eyes and ocelli with behavioral attraction of flies to reflective colors and patterns in light tunnel assays. Compound eye and ocellar electroretinogram responses to reflected light were similar, with the largest responses to white and blue followed by yellow, red, green, and black. However, data from light tunnel behavioral assays showed that flies were attracted to white and blue light but were repelled by yellow. The addition of a black line pattern enhanced the attractiveness of blue visual targets, whereas yellow lines decreased attractiveness. Sensory input from the compound eye and the ocellus seems to be integrated to direct fly behavior. There is a direct correlation of house fly attractiveness to visual targets and the intensity of electrophysiological response, except for the yellow targets, which repel flies despite of intense electrophysiological response.

**KEY WORDS** house fly, color vision, behavior response, electroretinogram, light tunnel assay

Visual stimulus is one of the most important determinants in fly behavior, because these insects have over half of the head comprised of two large compound eyes accompanied by a cluster of three simple eyes (ocelli). House flies rely on reflected sunlight to detect objects in their environment while flying, finding food, searching for harborage and resting areas. Reflected light enters the compound eyes or ocelli and stimulates photosensitive cells that trigger phototransduction, which converts light photons to electrical signals for the nervous system, sending signals to the insect's optic lobe for interpretation (Zuker 1995). Signals received by the optic lobe may elicit changes in fly behavior, such as attraction or repulsion.

Color vision is the ability to distinguish differences in wavelengths of light but not the intensity of light (Hilbert 1992), and generally is considered to be associated with compound eyes in insects. The ocelli are believed only to perceive differences of light intensity and are not capable of detailed image formation (Mizunami 1994). However,  $\approx 200$  photoreceptor cells are present within each house fly ocellus, and positive responses to dark vertical objects have been reported

when house fly compound eyes but not ocelli were blinded (Wehrhahn 1984).

Typically, electroretinogram (ERG) studies use a direct light source, such as a xenon bulb, to generate a narrow range wavelength to measure neural responses of insect eyes. Although ERG studies with direct light sources showed sensitivity of house fly compound eyes to ultra violet (UV) light (ranging from 340 to 370 nm), and blue-green light (ranging from 480 to 510 nm) (Bellingham 1994), there is disagreement on the effect of reflected colors and their visual attractants on house fly behavior (Geden 2006).

Compound eyes of *Musca* contain visual pigments that maximally respond to blue-green light with sensitivity from  $\approx 440$ –540 nm (Salcedo et al. 1999). Blue fabric targets with the maximum reflectance of 466 nm have been shown to be more visually attractive to house flies than white and black fabric targets (Geden 2006). This same type of blue visual target has also captured stable flies (Foil and Younger 2006). House flies are attracted to hanging cords and similar objects as resting locations (Fehn 1958) and tend to follow edges while foraging (Conlon and Bell 1991).

To further understand visual attraction of house flies to colors and patterns, and improve fly trap performances, the following studies were conducted to: 1) determine house fly physiological responses to reflective colors using ERG of both compound eyes and ocelli; 2) evaluate house fly behavioral attraction to reflected light from different colored substrates; 3) evaluate the effect of colored lines on attractiveness of a blue background.

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## Materials and Methods

**Insects.** *Musca domestica* (L.) used for these experiments were collected from the University of Florida Horse Teaching Unit and reared at the University of Florida, Gainesville, FL. Rearing rooms for all developmental stages were maintained at  $26 \pm 1^\circ\text{C}$  and 55% RH with a 12:12 (L:D) photoperiod. House flies were reared by placing eggs in a basin containing freshly mixed larval medium (1.5 liters of tap water, 250 ml Calf Manna pellets, Manna Pro Corp., St. Louis, MO, 15 ml methyl paraben, and 3 liters of wheat bran). Larvae were allowed 1 wk to pupate. Pupae were collected by flooding the rearing basin's content in tap water to cause the pupae to float. Pupae were collected and allowed to dry and then placed in a screened cage along with granulated sugar, powdered milk, and water ad libitum. After adult emergence, 3–5 d old house flies were collected by aspiration with a hand vacuum with a modified crevice tool.

**Electroretinogram.** Equipment similar to and techniques modified from Bellingham (1994) were used to measure depolarization of nerves in compound eyes and ocelli exposed to reflected light. Equipment consisted of two ERG micromanipulators (EAG COMBI 10X, Syntech, Kirchzarten, Germany) containing a  $10\times$  amplification of the recording electrode with glass sheathed tungsten electrodes to record depolarization of compound eyes and ocelli. Electrodes were electrolytically sharpened by etching with saturated potassium hydroxide. Signals from electrodes were further amplified (Autospike IDAC, Syntech) and recording software (ElectroAntennography Version 2.4, Syntech) on a computer was used to measure depolarization. To reduce external electrical interference, ERG equipment was enclosed in a Faraday cage and completely draped with black cloth to exclude light. Internal cage conditions during testing were  $24 \pm 1^\circ\text{C}$  and 45% RH.

**Visual Target and Light Source.** Visual targets of six different colors were used for ERG, and were constructed from twin-walled, rigid plastic sheets ( $20 \times 25$  cm; White C201L, Blue C505L, Black C208L, Yellow C402T, Green C406T, and Red C404T, Coroplast, ThyssenKrupp Material NA, Inc., Madison Heights, MI) (Beresford and Sutcliffe 2006). Visual targets were placed 25 cm in front of the house fly's head and arranged perpendicular to the floor.

The light source consisted of four warm white LED lights (RL5-WWW7035 2940K/700 mcd/ $35^\circ$ , Super Bright LEDs Inc., St. Louis, MO) mounted on an imprinted circuit board and fitted with a switch. The light source was powered by a 6V battery (BSL0955 6V 10Ah rechargeable battery, Universal Power Group Inc., Carrollton, TX) and held in place by a three-prong clamp at a maximum of 30 cm from the visual target. However, the light source distance from the color targets was adjusted so reflected intensity, as measured by USB2000 Spectrometer (see below), was maintained at the same level for different targets. A black piece of Coroplast was placed under the light source to prevent any light other than that reflected

from the visual target from reaching the test insect. All replicates were conducted in complete darkness.

Wavelength of reflective light from visual targets was measured using USB2000 Spectrometer (Ocean Optics, Dunedin, FL) with visual targets in place and the sampling optics of the spectrometer sensor (P600–2-UV-VIS, Ocean Optics) secured just above the test insect. Five independent readings were conducted for 1 min each. Light intensity of reflected light from visual targets was measured with a HOBO Light Intensity logger (Onset, Bourne, MA) in the same manner with HOBO device in place of test insect. Spectral reflectance of sunlight from colored plastic visual targets was also measured using the same equipment. Each visual target color was exposed outdoors to mid morning sunlight and the full spectrum of the reflected light was recorded.

**ERG Procedure.** Seventy female house flies (40 for compound eyes and 30 for ocelli) were used for this experiment and were anesthetized with ice for 30 min. Each fly thorax was encased with wax and adhered to a glass slide, ensuring no visual obstruction of the compound eyes or ocelli. The slide-mounted fly was clamped in place with the head facing the visual target. Electrodes were inserted into the appropriate eye using a dissecting microscope for precise placement. For compound eye bioassays, the measuring electrode was inserted into the equatorial region of the right compound eye (Bellingham 1994). For the ocelli bioassay, the measuring electrode was shallowly inserted, just puncturing, in the cuticle of the median ocellus, to avoid detecting neuro responses from interocular setae and the compound eyes. In both bioassays, the differential electrode was inserted into the fly's abdomen. After both electrodes were in place, the house fly was allowed 1–2 min to recover before bioassays.

Each fly was presented with a black, white, red, green, yellow, and blue visual target, allowing a 1-min recovery between each 0.5 s exposure of light source on the visual targets. During the 1-min recovery time, the color target was removed from the holder and replaced with a new target with different color. The ocellar ERGs were conducted separately from compound eye bioassays. Both experiments were run in a block design with each insect serving as a block, and being exposed to all colors in random order.

**Data Analysis for ERG Experiments.** Data from the ERG consisted of maximum depolarization measured in mV for the compound eyes and ocelli. One-way analyses of variances were performed on the compound eye and ocellar responses, with color as the main factor and the individual flies as blocks. Means were separated using Tukey's test ( $P = 0.05$ ; SAS 2001).

**Two-Sided Light Tunnel.** The two-sided light tunnel (TSLT) was constructed with two 43 liters capacity heavy-duty ice chests (Fig. 1) (The Hercules ice chest No. 5345; Life-Like Products Inc., Baltimore, MD). A circular hole (10 cm diameter) was cut in one of the side walls of each ice chest centered at 16 cm from the top, 17 cm from the bottom of the ice chest, and 29 cm from the sides of the ice chest. This hole was

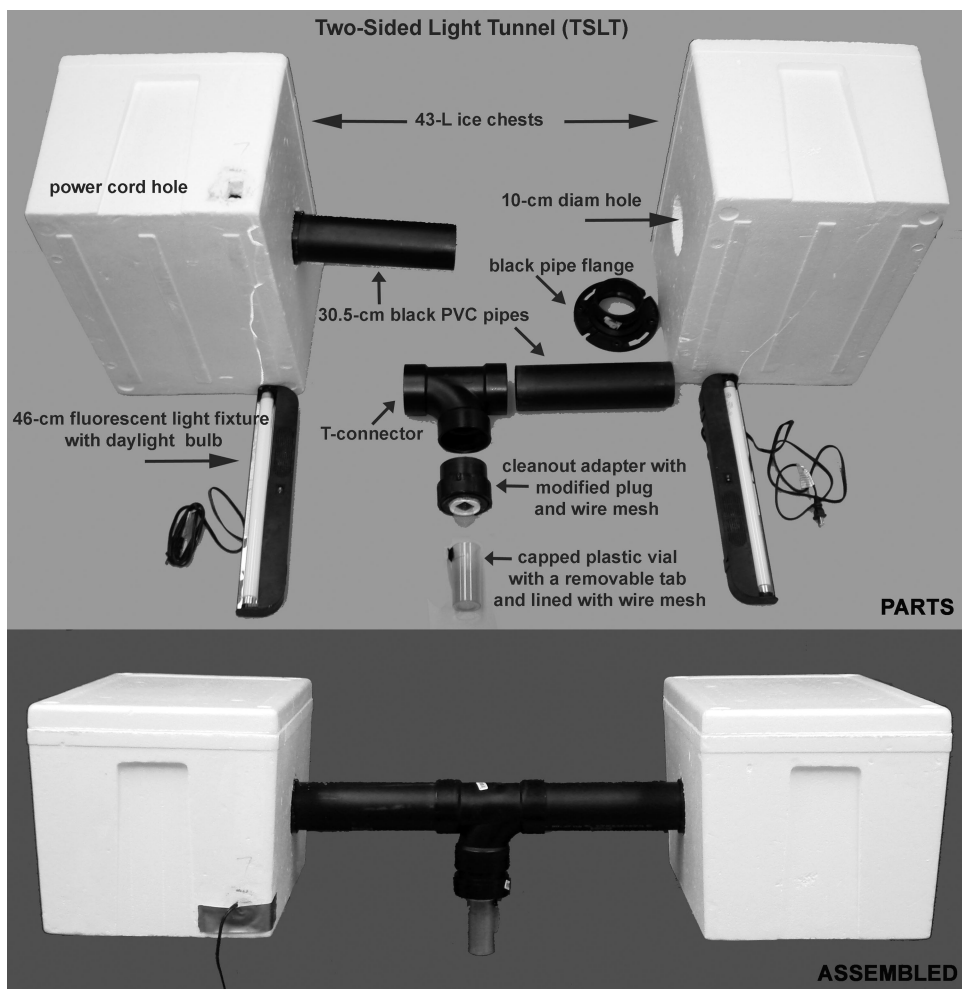


Fig. 1. TSLT parts (top) and assembled (bottom).

fitted with a black pipe flange (ABS-DMV Schedule 40; NIBCO, Inc., Elkhart, IN) secured in place with hot glue. Each ice chest contained a 46 cm fluorescent light fixture (Portfolio 18" under cabinet fluorescent light, model# GL9718-T8-BK-1, Good Earth Lighting, Inc., Wheeling, IL) centered directly under the black flange. The fluorescent light fixture was fitted with a daylight fluorescent bulb (GE daylight F15 457 mm, F15T8/D/TP, General Electric Company, Cleveland, OH) (Shields 1989). A square hole (3 cm side) was cut in the lower corner of the side wall of each ice chest to allow the power cord from the light fixture to exit the ice chest. The area around the power cord was sealed with modeling clay (Marblex Self Hardening clay, American Art Clay Co., Inc., Indianapolis, IN) to prevent house flies from escaping. Two ice chests were connected by two 30.5 cm black PVC pipes (TrueFit System 3300 3" SCH 40 COEX ABS cellular core DMV pipe Charlotte Pipe and Foundry Company, Charlotte, NC) which connected in the center to a T-connector (NIBCO). The T-connector was fitted with a cleanout adapter with a modified plug (NIBCO) so

that a capped plastic vial (50 dram Crystal capped plastic vial; Thornton Plastics, Salt Lake City, UT) could snap into the cleanout adapter and be removed when necessary. The snap-cap vial was modified with a removable tab that prevented flies from leaving the vial until the tab was pulled out. In addition, the snap-cap vial was lined with wire mesh that allowed the house flies to crawl up the sides of the vial to enter the TSLT. Similar wire mesh was also inserted in the modified plug and extended from the tab in the vial to the top of the cleanout adapter. This allowed the house flies to climb from the vial to the T-connector and enter the TSLT to make a choice between the ice chests with alternative visual targets.

**Visual Targets.** Fabric visual targets were constructed from Nautolex skipper fabric (20 × 25 cm, OMNOVA Solutions Inc., Fairlawn, OH) and plastic visual targets were constructed from twin-walled rigid plastic sheets (20 × 25 cm, Coroplast). Both fabric and plastic visual targets were yellow, red, green, black, blue, and white. Visual targets were centered on the inside wall opposite the pipe flange. Only the visual

**Table 1.** Wavelengths (nm) and intensity (cd/m<sup>2</sup>) of light reflected from visual targets used in electroretinogram and two-sided light tunnel experiments

Light source/assay <sup>a</sup>	Material	Color	Wavelength (λ)		Reflected intensity (cd/m <sup>2</sup> )
			Peak (nm)	Range (nm)	
Warm white LED/ERG	Plastic	Blue	460	423–574	11.8 <sup>b</sup>
	Plastic	White	500	419–738	11.8
	Plastic	Yellow <sup>c</sup>	564	510–702	11.8
	Plastic	Green	543	462–571	11.8
	Plastic	Red	603	588–702	11.8
	Plastic	Black	** <sup>d</sup>	**	11.8
GE daylight fluorescent/TSLT	Fabric	Blue	436	397–586	35.5
	Fabric	White	518	395–725	248.6
	Fabric	Yellow <sup>c</sup>	571	504–752	153.9
	Fabric	Green	544	450–571	28.0
	Fabric	Red	612	583–752	28.0
	Fabric	Black	**	**	19.4
	Plastic	Blue	482	394–583	59.2
	Plastic	White	522	380–812	232.5
	Plastic	Yellow <sup>c</sup>	575	502–738	106.6
	Plastic	Green	544	457–570	28.0
	Plastic	Red	610	578–738	19.4
	Plastic	Black	**	**	12.1

<sup>a</sup> ERG, electroretinogram; TSLT, two-sided light tunnel.  
<sup>b</sup> Light source distance from the color targets was adjusted so reflected intensity was maintained at the same level for different targets.  
<sup>c</sup> Yellow also reflected a peak in the non-attractive red region.  
<sup>d</sup> \*\*, no measurable peak or wavelength.

targets on each side could be seen from within the piping of the TSLT.

For experiments with lined visual targets, lines were added on blue visual targets by attaching 6 mm wide strips of skipper cloth spaced 1 cm apart and attached vertically on solid colored background. Vertical stripes were placed so that four lines could be seen when looking through TSLT from T-connector.

**TSLT Procedure.** All TSLT experiments were conducted with ambient temperature at 26.6°C. House flies (50 males and 50 females) were anesthetized with ice and placed in the modified snap cap vial. The snap cap vial was attached to the TSLT and the house flies were given 1 h to recover before removing the tab and allowing the house flies to enter the TSLT. To avoid any potential effects of position, because TSLT were stacked during the assays, experiments were conducted in a Latin square design with both TSLT position in the stack and daily replicate used as blocking factors. After fly release, each replicate ran 2 h. Once the allotted time was reached, the pipes were disconnected from the ice chests and all pipes and chest holes were immediately capped to prevent any fly escape and to separate flies in the ice chests from those remaining in the pipes. All flies were knocked down with CO<sub>2</sub> and counted. House flies that flew into the ice chests were considered responsive to the reflective color, whereas the flies that remained in the connecting pipes were considered nonresponsive.

Four TSLT experiments were conducted. In the first two experiments, individual color targets (white, red, yellow, blue, or green) were compared with a black target. The first experiment was conducted with fabric targets and the second one with plastic targets. For the

third experiment, the most attractive plastic targets from the first experiments (blue vs. white) and least attractive plastic target (yellow) were compared. This experiment was designed to provide direct comparison between white and blue targets, both against each other and the least desirable target (yellow). For the fourth experiment, colored lines (red, yellow, white, black, and no lines) were added to the blue target previously selected as the preferred color and again compared with a standard solid black target.

**Data Analysis for TSLT Experiments.** Total house fly response was calculated by subtracting nonresponsive flies (in piping) from total number of flies released in the TSLT. The proportion of house flies responding to individual visual targets was calculated by dividing the number of house flies collected from each of the two ice chest by the total responsive house flies. House fly responses to visual targets data were arcsine-square-root transformed before analysis of variance (ANOVA) and means were separated using Tukey's test ( $P = 0.05$ ; SAS 2001).

Results

**Visual Target Spectra.** Very little difference was observed between the reflected light spectra from the fabric and plastic targets used in our experiments (Table 1), with minimal differences when targets were illuminated with different light sources. The largest difference in the peak wavelength was for the blue targets with peak at 436 nm for fabric targets and 482 nm for the plastic ones. For the other colors, the difference between the peaks for plastic and fabric targets were only between 0 and 4 nm. In general, the



**Table 2.** Mean depolarization ( $\pm$ SE) in the equatorial region of right compound eye and median ocellus of female house flies stimulated with light reflected from plastic visual targets of different colors illuminated by a white LED light source

Visual target color	Compound eye response (mV)	Ocellus response (mV)
White	8.3 $\pm$ 0.97 a	4.9 $\pm$ 0.22 a
Blue	7.4 $\pm$ 0.97 ab	3.6 $\pm$ 0.17 b
Yellow	6.8 $\pm$ 0.94 b	3.0 $\pm$ 0.19 c
Red	5.2 $\pm$ 0.64 c	2.7 $\pm$ 0.21 c
Green	4.3 $\pm$ 0.49 cd	1.8 $\pm$ 0.12 d
Black	3.3 $\pm$ 0.39 d	1.7 $\pm$ 0.05 d

N = 40 for compound eye and 30 for ocellus assays.  
Means within columns followed by different letters were significantly different at  $P = 0.05$  (Tukey's test).

fabric targets reflected light in a wider range of wavelengths than the same color plastic targets. The yellow and white targets showed two peaks in the reflected light spectra, one around  $\approx$ 550 nm and the other at  $\approx$ 650 nm.

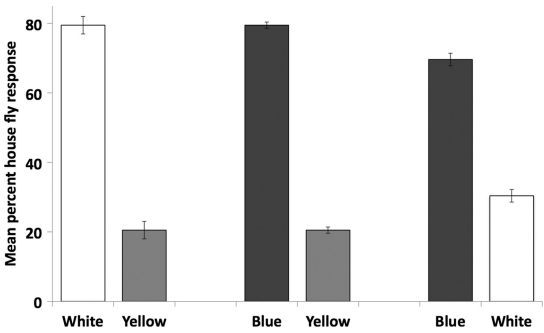
**Electroretinogram.** Extent of depolarization of compound eyes exposed to different color targets was significantly different ( $F = 30.99$ ;  $P < 0.0001$ ;  $df = 5, 75$ ) (Table 2). Responses to visual targets did not differ between white and blue, or between blue and yellow, but white caused significantly more depolarization than yellow. The other three colors (red, green, and black) caused significantly less depolarization than the first three colors. The ocellar responses were parallel to the compound eye responses, and showed similarly significant differences ( $F = 90.23$ ;  $P < 0.0001$ ;  $df = 5, 195$ ), with greatest depolarization for white and less for other colors (Table 2).

**Two-Sided Light Tunnel.** Responses of flies to different colored targets were significantly different (Table 3) ( $F = 20.97$ ;  $P < 0.0001$ ;  $df = 4, 25$ ), but no significant differences were observed between the responses of male and female flies (paired  $t$ -test;  $P = 0.57$ ;  $df = 29$ ). More flies were attracted to the white and blue fabric targets than to all other colors. Less than 50% of the responsive flies were attracted to the yellow target. With plastic visual targets, the house fly behavior was similar to that observed with fabric targets; however, the attraction of the red plastic target was significantly lower than that for the blue target, a difference that did not occur with fabric targets.

**Table 3.** Percent of house flies that responded ( $\pm$ SE) to colored fabric and plastic visual targets in two-sided light tunnel with GE daylight fluorescent bulb, when offered alternative black target

Target color	Fabric target	Plastic target
White	67.0 $\pm$ 3.86 a	67.3 $\pm$ 4.12 a
Blue	64.4 $\pm$ 3.17 a	66.4 $\pm$ 3.44 a
Red	57.3 $\pm$ 2.13 ab	55.3 $\pm$ 2.20 b
Green	48.2 $\pm$ 3.12 b	45.2 $\pm$ 3.61 b
Yellow	30.8 $\pm$ 3.81 c	32.9 $\pm$ 2.86 c

N = 6 for each combination of color and material.  
Means within columns followed by different letters were significantly different at  $P = 0.05$ . No differences were observed between the response by male and female flies (paired  $t$ -test;  $P = 0.57$ ;  $df = 29$ ).

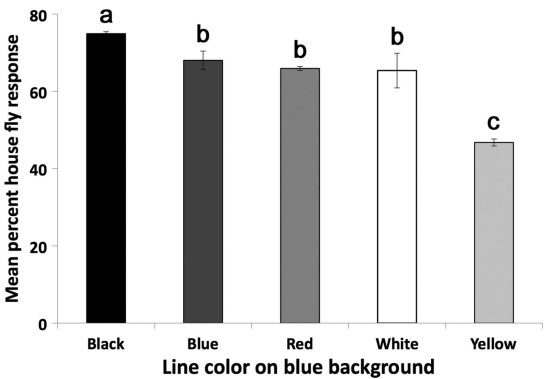


**Fig. 2.** Mean percent of house flies that responded to paired alternative fabric visual targets in two-sided light tunnel. Error bars represent standard error of the mean. Means within each pair of colors are significantly different ( $t$ -test;  $\alpha = 0.05$ ;  $N = 4$ ).

In direct comparisons, the white and blue targets attracted  $3\times$  more flies than were attracted by yellow targets (Fig. 2). In addition, the blue target attracted  $>2\times$  the number of flies attracted to white target. The addition of a lined pattern showed that black lines significantly enhanced the blue target's attractiveness (Fig. 3). The addition of red or white lines to the blue targets did not make them more attractive than plain targets with no lines. However, the addition of yellow lines made the blue target less attractive than the standard black target used in the TSLT assays.

**Discussion**

Reflected light from the color targets elicited an electrophysiological response in both the house fly compound eye and ocellus. Because the reflected light from all visual targets had the same light intensity, the variations in the ERGs in response to different colors were considered to be responses to wavelength, and not to light intensity.



**Fig. 3.** Mean percent of house flies that responded to blue targets with different colored lines in two-sided light tunnels compared with a solid black target. Blue treatment represents a solid blue target with no lines, and error bars represent standard error of the mean.  $N = 10$  for each colored line for a total of 50 runs.

The ERG showed that retinal cells in the house fly compound eye were highly depolarized by reflected light from white, blue, and yellow targets. White ( $\approx 419\text{--}738\text{ nm}$ ) and blue ( $\approx 423\text{--}574\text{ nm}$ ) reflected wavelengths that included the wavelength ranges included in the visual sensitivity for house flies, 450–510 nm (Agee and Patterson 1983, Bellingham 1994). The yellow visual target ( $\approx 510\text{--}586\text{ nm}$ ) caused similar physiological responses by the house fly despite the light wavelength being outside the vision range previously determined for this insect (Agee and Patterson 1983, Bellingham 1994). However, *Musca* flies have blue-green visual pigments in their compound eyes with sensitivity to wavelengths 440–540 nm (Salcedo et al. 1999), a range that partially covers the spectrum reflected by the yellow targets, and the presence of these blue-green pigments may explain yellow targets causing a neurological response in the compound eyes. Despite the response to yellow light, the low electrophysiological response to the green visual target, which reflected light waves (462–571 nm) that should be detected by the same blue-green visual pigments, was not significantly different from black target.

In other Diptera studies, shades of blue have been shown to be effective as visual attractants (Green and Flint 1986). Sunbrella blue fabric (peak wavelength 466 nm) was field-evaluated as a visual attractant in tsetse fly traps (Geden 2006, Mihok et al. 2006). Our results and these publications indicate that the material used as visual targets is not an essential factor in determining attraction to the flies, but the wavelengths of the target's reflected light is critical.

Visual pigments in fly ocelli have been shown to have a maximum absorption of blue light at 425 nm (Kirschfeld et al. 1988), which was reflected by white and blue visual targets. Ocelli can detect contrast, when the compound eyes are not functional, and this may facilitate locating dark vertical objects (Wehrhahn 1984). However, the ocelli are typically thought of as having high-speed neurons that only detect changes in light intensity (Chapman 2006). Visual input from the ocelli may provide sensory information for house flies to respond to visual targets.

Our behavioral experiments using the TSLT demonstrated that yellow was significantly less attractive verging on repellent to house flies, either as a solid color in comparison to white or blue or as lines on an attractive blue background. This contradicts previous experiments where more house flies landed on yellow paper than on papers of other colors (Awati and Swaminath 1920), being caught in yellow painted plastic jug traps (Burg and Axtell 1984), or being attracted to yellow cards (Nava 1967, Hecht et al. 1968). Wavelengths reflected by the traps used in those experiments were not reported by the authors. Comparison of attraction to certain wavelengths is impossible. However, visual targets may look very similar to human eyes but reflect wavelengths in very different ranges, especially if some of the reflected wavelengths are outside the range perceived by the human eye, but within the range perceived by *Musca domestica* com-

pound eyes and ocelli. Direct comparison of the two most attractive colors in the TSLT confirmed blue as more attractive than white (Fig. 2). Because white includes a wide range of wavelengths, including wavelengths peaking in the attractive blue and the repellent yellow range, white light may present the flies with conflicting stimuli.

Weather conditions such as air temperatures may influence the attraction of house flies to specific colors (Muniz 1967, Pickens 1995), and blue may be attractive to tsetse fly and other flies because it is perceived as shaded, cooler resting areas (Steverding and Troschianko 2003). The addition of black lines to the blue targets made them more attractive to house flies perhaps by adding stimulus to satisfy the scototaxis tendency of house flies (Hecht 1970). The house flies may perceive the blue visual target as a potential resting area and the black lines as cracks or crevices that can be used as harborage. It is possible that, under cooler conditions, the blue targets would not be as attractive as they were under the relatively warm experimental conditions used in our studies.

The visual information from the house fly compound eyes and the ocelli may be integrated to form sensory input that influences fly behavior in response to reflected light from objects. Our studies have shown that the ocelli are capable of color vision and thus may contribute more to house fly vision than originally thought. Comparison of the behavioral and physiological responses shows a direct correlation of house fly attractiveness to visual targets and the intensity of neurological response, except for the yellow targets, which triggered high electrophysiological responses but repulsion. These results represent an important step in the development of house fly traps that combine visual elements with other attractants.

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